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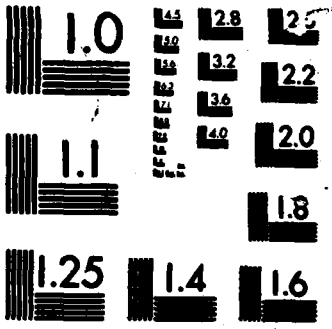


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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Our studies on the relatively simple nervous systems of the molluscs Pleurobranchaea and Aplysia have inquired into the neuronal basis of integrated behavior, the effect of learning on such integration, identification of neurons involved in the learned behavior, and small network modeling of learning. Significant findings are: (1) Neurocircuits establishing whole-animal behavior functionally emerge or "self-organize" within pools of coactive neurons; i.e., functional neurocircuits arise moreso from nonlinear dynamical properties than from static ("switchboard") anatomical ones. (2) Pharmacologic antagonists of cholinergic muscarinic receptors enhance one-trial Pavlovian conditioning. The specificity of this effect on associative processes provides an important inroad for experiments aiming to identify neurons involved in learning, and also to understand how learning biases self-organization. (3) In support of a goal-seeking theory of learning, conditioning of small nerve networks shows that cellular changes relating to an analog of learning involve postsynaptic processes in addition to presynaptic ones. These findings have broad implications in neurosciences and artificial intelligence.

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## A. OBJECTIVES AND STATUS OF RESEARCH

The scope of the research has been to determine the neuronal basis of learning in whole animals, and, in conjunction with this, to determine whether there are postsynaptic neuronal changes that occur in conditioning of identified neurons small nerve nets. Two different experimental preparations were used to experiment on these objectives. For the first objective, we began by looking at learning in whole animals of the sea slug Pleurobranchaea and then carried the analysis to the level of dissected preparations. Inasmuch as the definition of learning has been established on whole animals, it was necessary to establish quantitatively and qualitatively all the criteria of learning as they occur in whole animals and then compare them with those in behaving physiological preparations. The physiological indices of learning in these preparations would then form the defining criteria for comparison to even further reduced preparations. For the second objective, we used a known reduced preparation of the sea slug Aplysia (both of these animals and work on them are reviewed by Mpitsos and Lukowiak, 1985). The experiments on each objective were as follows:

### Objective I

a. Statement of the Problem:

Does learning persist during the dissection and electrophysiological preparation of the animals? And, is the traditional interpretation of neural activity as "switchboard" processes sufficiently apply to the findings?

b. Results:

1. We developed differential Pavlovian conditioning procedures for examining experimental and control responses in the same animal (Mpitsos and Cohan, 1986a,b).

2. We found that the learned behavior of the same animal(s) during electrophysiological recording was quantitatively and qualitatively the same as before dissection (Mpitsos and Cohan, 1986c); i.e., learning persists in the physiological preparations.

3. However, the electrophysiologically recorded motor patterns from trained and even from untrained animals exhibit considerable variability (Mpitsos and Cohan, 1986c). Variability has several implications. Technical implications: in multibehaving systems (ones capable of producing several different responses with the same muscles and neurons) it is not possible to identify reliably a behavior from electrically recorded motor patterns; i.e., for studies of whole-animal behavior, it is not possible to indefinitely reduce the physiological preparations, and to make comparisons of neuron function with behavior it is necessary to correlate the neuronal activity with observed behavior not with the recorded motor pattern. Theoretical implications: the "switchboard" theory of functional neurocircuits does not apply because different motor patterns can produce similar responses and, conversely, and different motor patterns can produce the same

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response. It is necessary to reformulate the conceptualization of integrative neural activity, as follows in (4).

4. The central nervous system of Pleurobranchaea has function distributed among many neurons, a central oscillator provides patterned activity to follower motor centers (although other neural oscillators may come into play secondarily), and there is some selective recruitment of neurons during the formation of different motor patterns (Cohan and Mpitsos, 1983a,b). However, many neurons are multifunctional in taking part in several motor patterns, and while it is possible to identify the types of connections between neurons, these connections can not be used to predict the overall output of the system: It is necessary, we propose, to view such nervous systems as having nonlinear dynamical properties that result in emergent or self-organizing activity (Mpitsos and Cohan, 1986d).

5. Therefore, before attempting to identify neurons in reduced preparation, it is necessary to identify from whole animal behavior neurons that become involved in learning. To do this we have developed biochemical probes using the cholinergic nervous system of Pleurobranchaea. First, antagonists such as scopolamine (as opposed to agonists such as oxotremorine) enhance one-trial Pavlovian conditioning, but do not seem to affect the normal nonconditioned behavior of the animals (Fig. 1 shows results of one experiment; Mpitsos, Murray, Creech, and Barker, 1986, are presently preparing a manuscript for publication detailing results of a complete experiment). Thus, cholinergic muscarinic receptors provide direct access to neurons taking part specifically in establishing associative learning. Second, we have demonstrated that the animals we study contain muscarinic receptors that have the classically defined pharmacological properties of those found in higher animals and humans (Barker, Murray, Siebenaller, and Mpitsos, 1986; Murray, Mpitsos, Siebenaller, and Barker, 1985). Third, we are presently developing fluorescent immunohistochemical and radiolabel techniques for visualizing and identifying the neurons that contain the muscarinic receptors. Fourth, the results of such biochemical studies will then be used to analyze changes occurring in these neurons during learning (preliminary evidence shows that there is upregulation of muscarinic receptors) and to determine how learning affects these neurons so as to bias the self-organizational process.

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Objective II

a. Statement of problem:

The first major demonstration of cellular changes relating to learning have been demonstrated in a simple reflex of the sea slug Aplysia. These findings show that the changes occur in presynaptic convergence between the pathways of the conditioned and unconditioned stimuli (CS and UCS, respectively)The common follower neuron does not appear to take part in the conditioning processes; that is postsynaptic processes do not appear to take place (see

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Mpitsos and Lukowiak, 1985, for a detailed review). Therefore, we asked of the same experimental system, as used to show presynaptic changes, whether postsynaptic changes do in fact take place. Our findings of muscarinic action in conditioning (described above) indicate that postsynaptic ones probably do take place. Although muscarinic receptors may not be involved in the Aplysia reflex, we nonetheless conducted experiments on Aplysia because of the identifiability of the neurons involved and because we wanted to contrast effects occurring both presynaptically and postsynaptically on the same synapse.

b. Experimental Set-Up:

Preparation: reduced Aplysia preparation consisting of siphon, mantle, gill, and visceral ganglion. Intracellular recordings from identified gill motoneurons LDG1 and L7. One cell receives experimental conditioning while the other cell receives control procedures; control versus experimental neurons reverse in different experiments.

Conditioning procedures: CS consists of a light tap to the siphon skin. UCS consists of either electrical shocks to the pedal nerve or strong tactile stimulation of the gill. CS-UCS separation of 3 sec or greater result in no associative conditioning. To implicate the postsynaptic neuron we depolarize either LDG1 or L7 for a period of time between the CS and UCS; the second motoneuron either is not depolarized or receives depolarization between conditioning trials. Therefore, within the same preparation we have an experimental and a control response. The conditioned response is the postsynaptic potential arising in the motoneurons in response to the siphon tap; the unconditioned response is the gill withdrawal.

c. Results:

An example of the results is shown in Fig. 2. The depolarization bridges the temporal gap between the CS and UCS, making effective associative conditioning in a temporal separation between the CS and UCS that is not effective without the depolarization. Therefore, a postsynaptic response is necessary for associative conditioning to occur. We have successfully repeated such conditioning in over a dozen preparations, however, many other preparations, either do not show conditioned changes or exhibit only sensitization in which both neurons increase the response to the CS. Although we have enough neuron pairs for publication, we are holding back on publishing the results until we understand the lack of conditioning and variability in the preparations that do not show good associative depolarization-induced conditioning. Nonetheless, the present findings are the first to show a postsynaptic effect in associative conditioning in Aplysia, and are consistent with A. H. Klopf's a view of conditioning as involving goal-directed processes in individual neurons (A.H. Klopf. (1982).

The Hedonistic Neuron. Hemisphere Publishing Corporation: Washington. pp. 140).

B. PUBLICATIONS

Barker, D. L., Murray, T. F., Siebenaller, J. F., and Mpitsos, G. J. (1986). Characterization of muscarinic cholinergic receptors in the crab nervous system. J. Neurochem. 46, 583-588.

(We used the crab because it provides an inexpensive tissue to develop and test techniques. Moreover, its nervous system allows for easier whole-mount visualization of fluorescently labeled neurons. Once having developed the various techniques we can apply them to sea slug nervous systems)

Cohan, C. S., and Mpitsos, G. J. (1983a). The generation of rhythmic activity in a distributed motor system. J. Exp. Biol. 102, 25-42.

Cohan, C. S., and Mpitsos, G. J. (1983b). Selective recruitment of interganglionic interneurons during different motor patterns in Pleurobranchaea. J. Exp. Biol. 102, 43-58.

Mpitsos, G. J., and Lukowiak, K. (1985). Learning in gastropod molluscs. In The Mollusca (ed. A. O. D. Willows). New York: Academic Press. pp. 95-267.

Mpitsos, G. J., and Cohan, C. S. (1986a). Discriminative behavior and Pavlovian conditioning in the mollusc Pleurobranchaea. J. Neurobiol. In press.

Mpitsos, G. J., and Cohan, C. S. (1986b). Differential Pavlovian conditioning in the mollusc Pleurobranchaea. J. Neurobiol. In press.

Mpitsos, G. J., and Cohan, C. S. (1986c). Comparison of differential Pavlovian conditioning in whole animals and physiological preparations of Pleurobranchaea: Implications of motor pattern variability. J. Neurobiol. In press.

Mpitsos, G. J., and Cohan, C. S. (1986c). Convergence in a distributed nervous system: Parallel processing and self-organization. J. Neurobiol. In press.

Murray, T. F., Mpitsos, G. J., Siebenaller, J. F., and Barker, D. L. (1985). Stereoselective L-[$^3$H]QNB binding sites in nervous tissue of Aplysia californica: Evidence for muscarinic receptors. J. Neurosci. 12, 3184-3188.

Mpitsos, G. J., Murray, T. F., Creech, C., and Barker, D. L. (1986). The cholinergic muscarinic antagonist scopolamine enhances one-trial Pavlovian conditioning in Pleurobranchaea. In preparation: to be submitted to J. Neurosci.

Abstracts:

Barker, D. L., Murray, T. F., Siebenaller, J. F., and Mpitsos, G. J. (1986). Characterization of muscarinic cholinergic receptors in crustacean nervous tissue. Soc. Neurosci. 11, 325.

Murray, T. F., Mpitsos, G. J., Siebenaller, J. F., and Barker, D. L. (1985). Demonstration of stereoselective L-[$^3$H]QNB binding sites in nervous tissue of Aplysia californica and Pleurobranchaea californica. Soc. Neurosci. 11, 481.

Unpublished Symposia/Scientific Meetings:

Winter Conference on Brain Research (1983) workshop on cellular, chemical, developmental, and self-organizational aspects of neural plasticity. Organized by D. L. Barker and G. J. Mpitsos, Oregon State University. Coparticipants: J. H. Byrne, University of Texas Medical School; S. B. Kater, University of Iowa, Department of Biology; G. Hoyle, University of Oregon, Department of Biology.

Western Nerve Net (1985), Santa Cruz, CA. Presentation on cholinergic muscarinic receptors in molluscs and crustacea by D. L. Barker, T. F. Murray, J. S. Siebenaller, and G. J. Mpitsos.

C. PROFESSIONAL PERSONNEL ASSOCIATED WITH THE RESEARCH

1. Principal Investigator: George J. Mpitsos; Hatfield Marine Science Center, Oregon State University, Newport, OR 97365.

2. Dr. David L. Barker was on the AFOSR contract with G. J. Mpitsos, now with Protein Databases, Inc.,405 Huntington Station, New York 11746. (916) 757-2434. Dr. Barker is continuing to collaborate with G. J. Mpitsos to help implement PDI resources in the AFOSR-funded research; see publications listed above.

3. Dr. Christopher S. Cohan, Department of Biology, University of Iowa, Iowa City, Iowa 52242; (319) 353-3780. Cohan and Mpitsos have collaborated on self-organizational processes and learning in Pleurobranchaea throughout tenure of AFOSR-funded research; collaboration ended March, 1986. See publications listed above.

3. Clayton Creech, Research Associate with G. J. Mpitsos, computer programmer, June, 1981 to present; Mr. Creech is supported by the AFOSR contract, and is an integral member of the laboratory in the capacity of data analysis and experimental design.

3. Dr. Stanley B. Kater, Department of Biology, University of Iowa, Iowa City, Iowa 52242; (319) 353-3780. Research collaboration between Dr. Kater and G. J. Mpitsos was from June, 1983, to September, 1985, on application of conditioning/stress-related effects on growth of identified neurons; collaborative research may also occur periodically in the future.

4. Dr. Ken Lukowiak, Faculty of Medicine and Department of Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1, (403) 220-4493. Lukowiak and Mpitsos have been collaborating on a continuing basis since November, 1983, on conditioning of identified neurons in small nerve networks of Aplysia; e.g., see Fig. 2.

5. Dr. Thomas F. Murray, Hatfield Marine Science Center and Department of Pharmacology, Oregon State University, Newport, Or 97365; (503) 867-3011. Dr. Murray is a neuropharmacologist. Collaborative research with G. J. Mpitsos has been a continuing basis since June, 1982; see publications listed above.

6. Dr. Joseph F. Siebenaller, Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803; (504) 388-1132. Dr. Siebenaller is a protein biochemist. Collaborative research with G. J. Mpitsos has been on a continuing basis since June, 1982; see publications listed above.

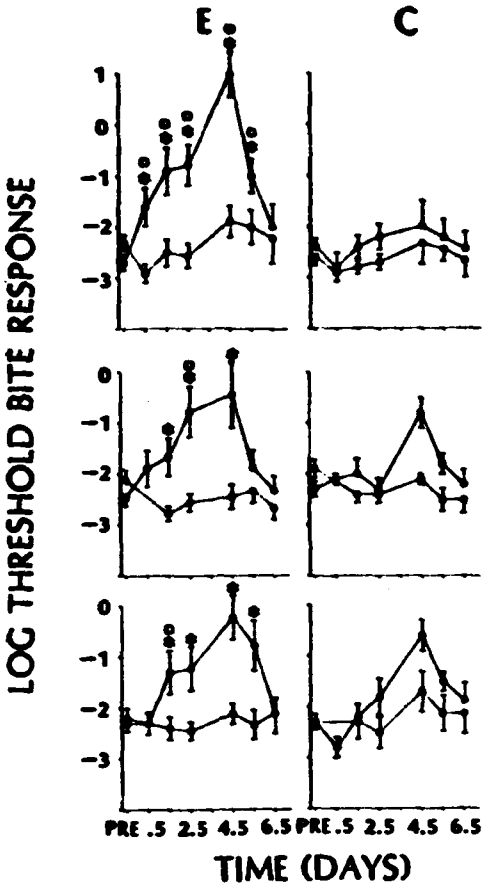
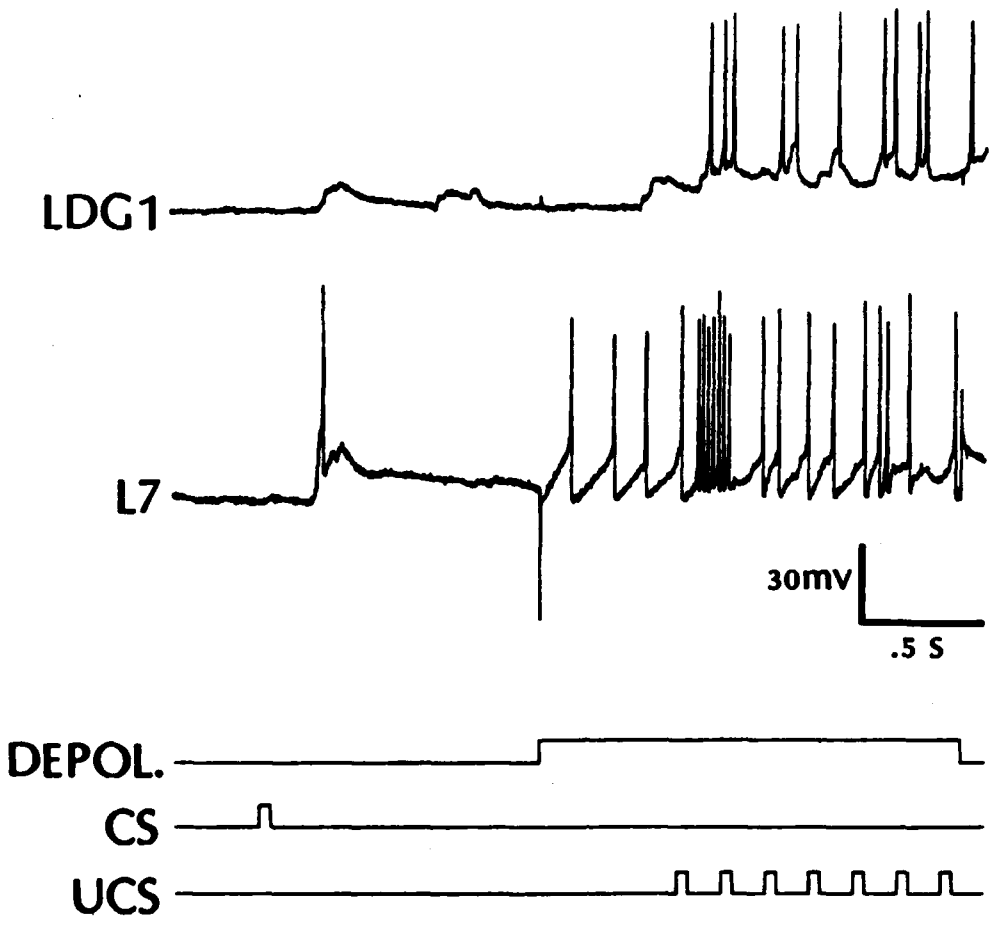



FIGURE 1. Scopolamine enhances the ability of animals to exhibit suppressed food-avoidance preferentially to a beer-derived stimulus (Sbr; upper curve of each pair of curves) than to a squid-derived stimulus (Ssq; lower curves). Curves show pre and postconditioning responses of three groups of experimental (E) and control (C) animals (10 animals in each E and C group; total N = 60). Preconditioning THRESHOLD TESTS (shown at PRE) were made the day before conditioning; postconditioning measurements began 12 hrs (.5 days) after conditioning and repeated every 24 hrs thereafter; measurements were not made on day 3.5. THRESHOLDS represent the logarithm of stimulus concentrations that elicited threshold responses; e.g., -1 is ten-fold stronger than -2. DURING TRAINING, Es received 20 ml of the -1 concentration of Sbr (CS) for 60 sec, the last 50 sec of which overlapped with electrical shocks (UCS) (see Mpitsos et al., 1978, for many of the procedures). The Cs received the electrical shocks 1 hr apart from the Sbr. We trained animals on only one day and for only one trial; by appropriate one-minute staggering of the procedures, we were able to train all animals concurrently. DRUGS: One set of Es and Cs (upper pair of illustrations) received an intraperitoneal injection of scopolamine 1 hr before training; another set received an injection of oxotremorine (middle pair of illustrations); and a third received an injection of the water vehicle (bottom pair of illustrations). Previous experiments on other animals showed that the injections did not affect the animals performance; i.e., their thresholds were the same after the injections as before. STATISTICS. Mann-Whitney U tests; significant differences are at $P \leq .05$. RESULTS. The scopolamine Es showed consistently significant differererences between their responnses to Sbr and Ssq: compare top curve with bottom curve; asterisks show statistically significant differences. These same Es showed consistent differences in their responses to Sbr with respect to the responses of Cs to Sbr: compare top curve on the E-side with top curve on the C-side; squares show statistical differences. Other Es and Cs did not show such consistent differences. Data represent means $\pm$ S. E.



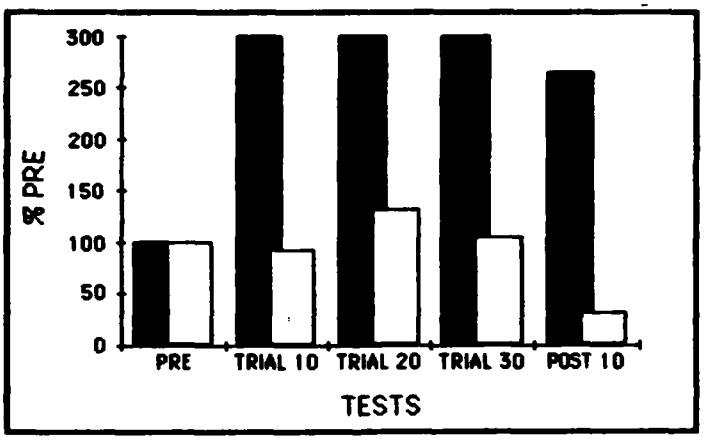




FIGURE 2. Upper traces show conditioning of two Aplysia gill motoneurons. During training, the experimental neuron (L7 in this case) receives the CS and UCS plus depolarizing current passed through the recording microelectrode. The control neuron (LDG1) receives the two conditioning stimuli but no depolarization. Before training neither neuron fired to the CS, but during training (shown here is Trial 3 in the first block of 10 trials) only L7 fired, and its EPSP occurring in response to the CS increased to about 200%. Bottom three traces show monitors of the the conditioning stimuli. In these experiments, the depolarizing stimulus overlapped with the UCS. Histogram shows the pre and postconditioning responses to tests with the CS alone; percentages are with respect to each neuron's EPSP amplitude that occurred before training. The postconditioning responses are for the tenth trial in each of three ten-trial conditioning sessions. Within each session, the intertrail interval was 5 min.; 40 min. rests separated each conditioning session. The "POST 10" test shows the EPSP percentages occurring to the CS on the tenth trial after the last conditioning session; as during conditioning, the interval between each post-test was 5 min. Note that the EPSPs of L7 increased during conditioning and persisted at the increased level during the postconditioning tests, whereas those of LDG1 remained at the preconditioning level during conditioning and habituated during the post tests.

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